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14. ABSTRACT:
We have designed and synthesized a novel compound (11β) that efficiently triggers apoptosis in prostate cancer cells such as LNCaP. This bifunctional compound was designed to form DNA adducts that are camouflaged by the androgen receptor making them less readily repaired in AR+ prostate cancer cells. The aims of our studies are to investigate the mechanisms by which 11β is able to trigger apoptosis in target cells. Methods have been developed that permit us to determine the fates of 11β-DNA adducts in treated cells in culture as well as in tumors growing in animal models. Another objective is to identify the signaling events that lead from DNA adducts to activation of the apoptotic program. Finally we have obtained encouraging results from animal experiments that indicate that molecules such as 11β may have clinical potential for the treatment of human tumors.

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Chemotherapy, DNA damage and repair, Apoptosis, Steroid Receptors

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INTRODUCTION

The objective of our research is to develop more effective therapeutics for the treatment of prostate cancers. One novel bifunctional compound (**11β**) that we have prepared rapidly induces apoptosis in several prostate cancer cell lines in vitro. The **11β** compound contains a chemically reactive nitrogen mustard linked to a steroid moiety that binds with high affinity to the androgen and progesterone receptor proteins. This compound was designed to create DNA adducts that form tight complexes with these steroid receptors; the physical presence of the receptor on the adducts makes the adducts difficult to repair in prostate cancer cells. Preliminary studies of **11β** in cell culture indicated that its effects on prostate cancer cells were distinct from those of other alkylating agents used in chemotherapy. The apoptotic responses of prostate cancer cells suggested that the **11β** compound might be a useful agent for chemotherapy, and our project explores that possibility. The Specific Aims of our research are to understand the fate of **11β**-DNA adducts in treated cells and to investigate the mechanisms that lead to apoptosis. We also proposed experiments to assess the antitumor potential of **11β** in animal models of human prostate cancer and to investigate the effects of our new compound in additional animal models of prostate cancer.

BODY

Task 1: *Determine if the biochemical changes observed in prostate cancer cells in culture are responsible for the antitumor effects of 11β in xenograft tumor models*.

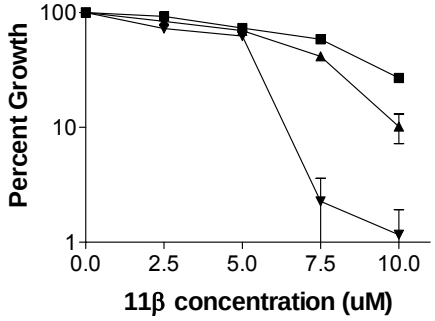

Figure 1. Toxicity of 11b towards AR- (PC3 -■-; DU145 -▲-) and AR+ (LNCaP -▼-) prostate cancer cells.

One biochemical change that we proposed as a marker of antitumor response was the DNA damage induced protein p21. During the past year we have examined the p21 response to 11β in a number of prostate cancer cell lines in addition to LNCaP including DU145 and PC3. These studies are near completion and we anticipate that they will provide a robust marker for biological response to 11β that will increase the reliability of our mechanistic studies in animal models. We have also characterized the cytotoxic effects of 11β in the DU145 and PC3 cell lines. Since both of these cell lines can be grown as xenografts in nude mice our results we can test whether the p21 responses observed in cell culture are similar to those in tumor models *in vivo*.

Task 2: *Determine the fate of 11β-DNA adducts in prostate cancer cells and in LNCaP xenograft tumors in animals.*

During the previous grant period we developed a highly sensitive method for the analysis of 11β DNA adducts using the technique of Accelerator Mass Spectrometry. Using a

radiolabeled analog that incorporated one 14C atom into the linker of our molecule we have been able to detect and quantify [14C]-11β-DNA adducts in treated cells. This permitted us to define the dose-response relationship between the concentration of 11β in cell culture media and the level of DNA adducts in cells. Since the cytotoxic effects of 11β in LNCaP cells are well defined we were able to obtain a relationship between DNA adduct levels and toxicity. (Fig 2). We then compared the DNA adduct levels in LNCaP xenograft tumors growing in mice after the animals were treated with [14C]-11β at doses that produced an antitumor response. These experiments revealed that the adduct levels achieved in xenografts after a single dose of 11β (0.14 to 0.24 11β-DNA adducts/10^6 bases) were within the range of those that showed toxic effects in our cell culture based experiments. Based on these results we think that 11β-DNA adduct levels will provide a useful metric with which to correlate toxicity *in vitro* with antitumor responses *in vivo*. Over the next year we will develop similar relationships for other prostate tumors to test the strength or this relationship. It also provides us with a facile means by which to evaluate to potential therapeutic effects of different 11β formulations, dosing schedules and routes of administration.

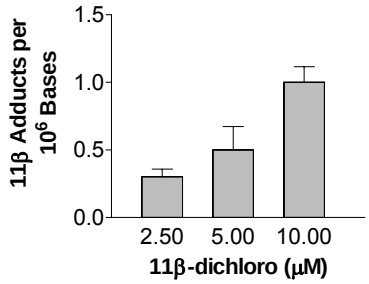

Figure 2. Formation of 11β-DNA adducts in LNCaP cells treated with [14C]11β for 2 hr.

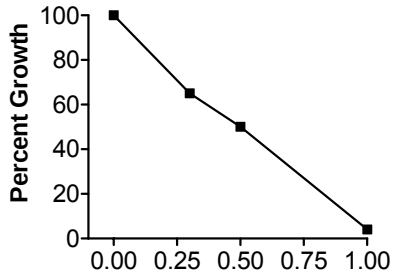

Figure 3. Relationship between the level of 11β DNA adducts and percent growth of LNCaP cells in culture.

Task 3: *Formulation of 11β-dichloro in a liposomal vehicle and investigation of its PK and efficacy*. As the result of personnel changes, we have had to postpone the start of this portion of the project. Dr. Shawn Hillier, the graduate student who began this work left our laboratory after receiving his doctoral degree in May 2006. This was a great success for Dr. Hillier and our program but it left us without key expertise to begin this task. A new graduate student, Mr. Frances Gonzoles has started to gain experience with animal models and we anticipate that he will begin work on this task during the next year.

During the next year we shall continue to investigate the pharmacokinetics of 11β in liposomes and assess its delivery to tumor and normal tissues in a xenograft mouse model of prostate cancer using [^{14}C]-11β. Traditional liquid scintillation counting methods will be used to determine the amount of 11β in each tissue, while the much more sensitive AMS analysis will be used to quantify 11β-DNA adducts that are formed in target and nontarget tissues. The measurement of DNA adducts will provide a direct assessment of the amount of biologically active compound reaching the desired molecular target within the tumor and allow us to investigate dosing parameters (e.g., amount, frequency and

route of administration). We will also seek to optimize the dose and schedule of administration to enhance the antitumor responses.

Task 4: *Assess the antitumor activity of 11β in additional human prostate tumor models*.

As reported in our FY 2004 Progress Report, the 11β compound is very effective in preventing the growth of LNCaP cell in mouse xenografts. It is especially encouraging that the dose of 11β that was effective in preventing tumor growth did not show significant toxic effects in the animals.

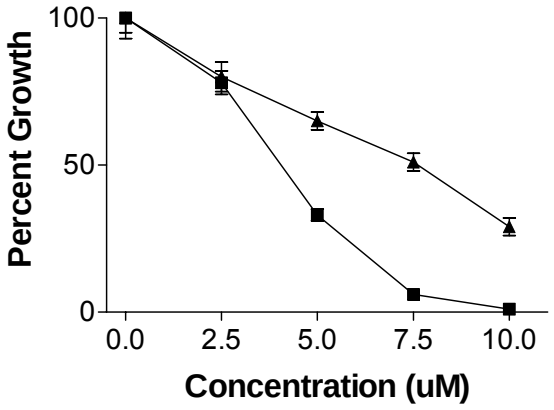

Figure 4. Toxicity of 11β towards PC3-neo (-▲-) and PC3-AR (-■-) cells.

We have initiated a set of experiments to characterize the antitumor activity in other prostate cancer models by characterizing the toxicity of 11β in several additional prostate tumor cell lines including PC3 and DU145. In addition, we have obtained a set of cell lines that will be useful for investigating the role of the AR in antitumor responses in xenograft models. These included derivatives of the PC3 cell line named PC3-neo and PC3-AR. These two genetically engineered cell lines are isogenic except for AR status. As shown in Figure 3, the presence of the AR increases the sensitivity towards 11β. We are evaluating these cell lines further in a parallel project that is focused more on mechanistic studies. This pair of cell lines may provide a more defined model with which to evaluate the role of the AR in tumor responses than the CWR22 and CWR22R cell lines described in our proposal. During the next year we will test the ability of PC3-neo and PC3-AR to form tumors when injected subcutaneously into nude mice. If these cell lines do grow as xenografts, this model will allow us to determine the role the AR in antitumor responses towards the 11β compound in vivo.

KEY RESEARCH ACCOMPLISHMENTS

- We have characterized the toxicity of our novel antitumor compound 11β towards several prostate cancer cell lines and examined the response of p21 as a potential biomarker of antitumor effects *in vivo*;
- Established the relationship between the level of 11β-DNA adducts formed in LNCaP cells in culture and cytotoxicity;
- Developed methods to measure the levels of 11β-DNA adduct in tumors of treated animals;
- Initiated mechanistic studies with isogenic cell lines that differ in AR status to determine the role of the AR in both the cytotoxic effects of 11β *in vitro* and tumor responses *in vivo*.

REPORTABLE OUTCOMES

Publications: Hillier, S.M., Marquis, J.C., Zayas, B., Wishnok, J.S., Liberman, R.G., Skipper, P.L., Tannenbaum, S.R., Essigmann, and Croy, R.G. DNA adducts formed by a novel antitumor agent 11β-dichloro in vitro and in vivo. 2006 Mol. Cancer Ther. 5(4); 977-984.

Degrees Supported:

- Dr. Shawn Hillier has been supported on this grant. He received his Ph.D. in June, 2005. Dr. Hillier was responsible for DNA repair and antitumor studies. He is now employed by Molecular Insight Pharmaceuticals, Cambridge, MA.
- Dr. John Marquis has received support from this grant. He recently left the lab and is now employed by Molecular Insight Pharmaceuticals, Cambridge, MA.
- Mr. Frances Gonzales is a graduate student who has been supported on this grant. He will defend his thesis proposal dealing with this work in May.

CONCLUSIONS

We are now engaged in experiments to define the amounts of DNA damage produced by the 11β compound and the fates of this damage in target and non-target cells in culture and tumor xenografts. This information will help us establish the sequence of events that lead to apoptosis. The results we have obtained in animal models are especially significant. The ability of the 11β compound to prevent the growth of LNCaP cells in a mouse xenograft model provides evidence of the potential clinical activity of this compound. The activity of 11β will now be tested in additional xenograft models of human prostate cancer to assess the range of its antitumor properties.

Our research during the next year will focus on the following goals: (1) continuing our experiments to identify and validate biomarkers such as p21 and the formation of 11β-DNA adducts that can be used to calibrate tumor responses to 11β in animal models; (2) initiating studies on reformulation of 11β focusing on liposomes as possible delivery vehicles for the compound and; (3) examining antitumor responses to 11β in other animal models of prostate cancer. During the past year we have characterized the toxicity and responses of AR+ and AR- cell lines towards 11β and we are now set to initiate our *in vivo* studies. We anticipate that identification of the signaling events originating from DNA adducts will provide valuable biomarkers of antitumor responses as well as clues to the reasons why the 11β compound is able to trigger apoptosis while other aniline mustard compounds such as chlorambucil do not.

REFERENCES

None included.

APPENDICIES

None included.